patient the dose of 4-HC has varied from 20 uM to 60 uM. At the end of incubation the marrow cells were washed one time and committed stem cell assays wen done just before freezing step. The isolation of bone marrow mononuclear cells using Ficoll-metrizoate with IBM 2991 appears as the method the best adapted for an in vitro treatment. Two patients have been grafted with b.m. processed in this way (the CFU-GM inhibition was significant = 8 and 20 % of recovery), nevertheless the engraftment time was not significantly delayed.

21.

MONOCLONAL ANTIBODIES ATTACHED TO MICROSPHERES CONTAINING MAGNETIC COMPOUNDS, USED TO REMOVE NEUROBLASTOMA CELLS FROM BONE MARROW TAKEN FOR AUTOLOGOUS TRANSPLANTATION.

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In stage 4 neuroblastoma (Evans' Classification), where cells can metastasise to bone marrow, the use of high dose chemotherapy, with autologous marrow transplantation as a therapeutic regime, carries the risk of reinfusing untreated tumour cells to a patient. Using monoclonal antibodies chosen for their binding to neuroblastoma and not normal bone marrow, we have investigated different approaches to the selective removal of tumour cells from autograft marrow. To date the optimum system involves the use of polystyrene microspheres (2μ diam.) containing 27% wt/wt magnetite and coated with affinity purified goat anti-mouse Ig. Beads coated with anti-mouse Ig will bind to cells incubated with mouse monoclonal antibodies directed against cell surface antigens. When placed in a magnetic field cells binding beads are drawn to the side of the tube leaving unlabelled cells in suspension. To initially model the removal of tumour from bone marrow, the human neuroblastoma cell line CHP 100 was added to different proportions of the leukaemic line Nalm-6 (ratios 1:1 to 1:10). To account for the antigenic heterogeneity observed in neuroblastoma a panel of monoclonal antibodies was added to the mixture. Following washing, cells were incubated with goat anti-mouse Ig coated beads and placed in a magnetic field. 97-99% of neuroblastoma cells could be removed from the suspension without non-specific trapping of Nalm-6. Similar results have been obtained titrating CHP 100 cells into normal bone marrow. Our current experiments suggest the methodology can be scaled up to separate malignant cells from 5 x 10^9 nucleated bone marrow cells.

22.

PURGING NEUROBLASTOMA (NB) CELLS FROM BONE MARROW (BM)

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Various in vitro methods have been used to purge the BM of malignant cells remaining after systemic treatment. We investigated the effect of 6-hydroxydopamine (6-OHDA) on neuroblastoma cells and normal BM. Five human NB cell lines were used. 6-OHDA at 20 ug/ml was found to be most effective and the effect was enhanced with Ascorbic Acid (C) at 100 ug/ml. In vitro incubation of 6-OHDA+C for one hour was 100% cytotoxic at cell concentrations below 7 NB cells/mm^3; at 7-12 NB cells/mm^3 only 0-2% survived. At concentrations of 20 ug/ml 6-OHDA and 100 ug/ml C there were no decreases in CFU-C of various BM's tested (14 samples) while concentrations of 6-OHDA greater than 40 ug/ml were toxic to BM CFU-C. 6-OHDA at 20 ug/ml does inhibit the BFU-e of BM, however, there is no correlation of BFU-e inhibition and subsequent ability for BM engraftment. Two-fold augmentation of specific NB cell kill in vitro by 6-OHDA-C plus 0.12 ug/ml Tropolone, a catechol orthomethyl transferase inhibitor was observed.

Two patients with disseminated NB and residual BM involvement had their BM purged with 6-OHDA+C. The BM's were reinfused after high dose Melphalan, dianhydrogalactitol and total body irradiation. Mild transient hypertension in one patient and diarrhea and mucositis in both patients were noted. Hematopoetic recovery and tumor regression were noted but the follow-up is short at this time and will be discussed. In vitro purging of tumor cells has an important role in the success of autologous stem cell transplants for patients with disseminated neuroblastoma.

23.

HIGH. DOSE CYTOREDUCTIVE REGIMEN FOLLOWED BY AUTOLOGOUS BONE MARROW TRANSPLANTATION (A.B.M.T.) IN CHILDREN WITH ACUTE LEUKEMIA.

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We have treated two children with acute lymphoblastic leukemia (A.L.L.) in relapse or in second remission, and three children with acute myelogenous leukemia (A.M.L.) in complete remission (C.R.) with high dose cytoreductive regimen followed by A.B.M.T. The T.A.C.C. regimen has been used in three children with A.M.L. in remission and in one child with A.L.L. in second relapse. The T.A.C.C. regimen consited of 6- Thioguanine = 400 mg/m2 daily by mouth on day 2 to 5, Cytosine-Arabinoside = 400 mg/m2 daily I.V. on day 2 to 5, C.C.N.U. = 400 mg/m2 by mouth on day 1 and Cyclophosphamide = 50 mg/kg I.V. on day 2 to 5. The A.B.M.T. is transfused two days after the last dose of Cyclophosphamide.

For one child with A.L.L. in relapse, this chemotherapy has failed to obtain a complete remission. Two children with A.M.L. grafted in first C.R., remain in remission for 16 months + and 2 months + without any maintenance treatment. One child grafted in second C.R. of A.M.L. relapsed six months after A.B.M.T., and he went into third C.R. after high dose melphalan (H.D.M. = 200 mg/m2) followed by bone marrow harvested two months before ; actually he is alive and well in C.R. for five months after the second A.B.M.T.

For one child with A.L.L. in second C.R., we used Cyclophosphamide (60 mg/kg/d. x 2 days) and T.B.I. (grays) followed by A.B.M.T. He remains in C.R. for fifteen months + without any maintenance treatment.

24.

THERAPY OF DISSEMINATED NEUROBLASTOMA WITH INTENSIVE THERAPY AND AUTOLOGOUS STEM CELL RESCUE.

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Autologous stem cell transplantation (ASCT) using cryopreserved bone marrow (BM) can be used to circumvent the hematopoietic toxicity of high dose chemotherapy. Two patients with extensive neuroblastoma were managed with 4-6 courses of conventional chemotherapy (N4SE); the patients had residual disease but the BM was not involved. The BM was then cryopreserved and patients were given high dose chemotherapy with melphalan (L-PAM) and dianhydrogalactitol (DAG) at a total dose of 180 mg/M^2 each over 3 days. Patients also received local radiation therapy to bulky disease. The cryopreserved BM was reinfused 48 hrs later. Both patients had good hematopoietic recovery within 17-28 days, and remain disease free 5 months later. Two other patients with disseminated neuroblastoma and BM involvement after conventional chemotherapy had their BM withdrawn and purged with 6-hydroxy-dopamine (6-OHDA) at 20 μg/ml and ascorbic acid (C) at 100 μg/ml for 1 hr. This combination is known to be a selective killer of neuroblastoma cell lines, without causing a decrease in BM CFU-c activity. Both patients then received L-PAM+DAG with total body irradiation (450 rads). Two days later, cryopreserved, purged BM was reinfused, mild transient hypertension (6-OHDA related) was noted. Hematopoietic recovery and tumor response was noted. The follow-up is too short to assess long term benefit. The toxicity of the above treatment includes nadir sepsis, mucositis, and diarrhea. Supportive care includes total parental nutrition, antibiotics and irradiated blood products to prevent graft versus host disease. We feel that early intensive chemotherapy with ASCT rescue has a place in therapy of disseminated neuroblastoma.

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25.

AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) IN THE THERAPY OF ADVANCED MALIGNANT TUMORS OF CHILDREN AND ADOLESCENTS.

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